Controlled reoxygenation during cardiopulmonary bypass decreases markers of organ damage, inflammation, and oxidative stress in single-ventricle patients undergoing pediatric heart surgery

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Objective: Single-ventricle patients undergoing pediatric heart surgery are a high-risk group owing to reoxygenation injury during cardiopulmonary bypass (CPB). The present study investigated the effects of controlled reoxygenation CPB on biomarkers of organ damage, inflammation, stress, and long-term functional outcomes in cyanotic patients with either a single or double ventricle during open heart surgery.

Methods: Cyanotic patients with either a single (n = 32) or double (n = 47) ventricle undergoing surgical correction were randomized to receive CPB using either standard oxygen levels or controlled reoxygenation. The markers of cardiac injury, inflammation, stress, and cerebral and hepatic injury were measured preoperatively, at 10 and 30 minutes after starting CPB, and at 10 minutes and 4 and 24 hours after CPB. The data were analyzed using a mixed regression model.

Results: No difference was found in the pre- or intraoperative characteristics between the standard and controlled reoxygenation CPB groups for single- or double-ventricle patients. In the single-ventricle patients, controlled reoxygenation CPB significantly ($P < .05$) decreased the markers of organ damage, inflammation, stress, and oxidative stress. In contrast, the markers of inflammation and cardiac injury were not altered by controlled reoxygenation CPB in the double-ventricle patients.

Conclusions: Controlled reoxygenation CPB decreased the markers of organ damage, stress, inflammation, and oxidative stress in single-ventricle patients undergoing cardiac surgery. (J Thorac Cardiovasc Surg 2014;148:792-801)

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A functional single ventricle describes an array of heart malformations with a single ventricular pumping chamber.[1] Although a variety of diverse anatomies exist (eg, hypoplastic left heart syndrome, tricuspid atresia, and double-inlet left ventricle), children with these malformations are exposed to very similar pathophysiologic states. The functional single-ventricle palliation often includes 3 operations. The first stage of palliation is performed at birth. The second stage is a bidirectional Glenn operation, usually undertaken at 6 to 8 months of age. The third, and final, stage is the Fontan operation, which can be performed between 18 months and 4 years of age. These infants and children are at a very high risk of developing perioperative and long-term complications that could affect their quality of life.[1,2] Additionally, a single ventricle has been considered a risk factor for operative mortality,[3] although this remains debatable.[4]

Reintroduction of high oxygen levels to cyanotic patients when starting cardiopulmonary bypass (CPB) leads to reoxygenation injury with significant organ damage, including the myocardium and triggering of a systemic inflammatory response.[5-7] One of the strategies proposed to avoid reoxygenation injury has been the use of controlled reoxygenation using a partial pressure of oxygen in arterial blood (PaO_2) similar to the patient's preoperative oxygen saturation when starting CPB. This has been shown to ameliorate reoxygenation injury in experimental models,[8,9] in adult patients,[10] and, more recently, in cyanotic pediatric patients with mixed

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Abbreviations and Acronyms

C3α = complement activation
CI = confidence interval
CPB = cardiopulmonary bypass
IL = interleukin
MIF = microphage migration inhibitor factor
PaO_2 = partial pressure of oxygen in arterial blood
TnI = troponin I

pathologic features who are undergoing cardiac surgery.[11] However, the degree and nature of cyanosis will vary depending on the individual pathologic features, with no studies comparing the efficacy of this intervention in cyanotic patients with different pathologic features and different risk stratification. Of particular interest are patients with a single ventricle who are at a greater risk of developing reoxygenation injury and its deleterious effects on multiple organ function. These patients have chronic cyanosis and have a relatively short cardioplegic arrest; thus, it has been easier to identify CPB-induced reoxygenation as the main culprit triggering organ injury and systemic stress. In the present study, we investigated whether controlled reoxygenation when starting CPB would alter the markers of organ damage, stress, inflammation, and oxidative stress in cyanotic patients with a single or double ventricle when undergoing cardiac surgery.

METHODS

The patients in the present study were a part of a large trial (study no. CS/2007/2678, recruitment just completed) investigating the effect of controlled reoxygenation CPB in cyanotic children undergoing surgical correction at the Bristol Royal Hospital for Children. The patients were randomized to receive CPB with either a standard oxygen partial pressure (150-200 mm Hg, hyperoxic to patients) or controlled oxygen partial pressure (matched to patients, normoxic). Data from a group of patients in the initial phase of recruitment have been published without any reference to the specific pathologic features or clinical outcomes.[11] The present study was a subanalysis of the effect of controlled reoxygenation in patients (including those from the first report) with either single (n = 32) or double (n = 47) ventricular pathologic features and included long-term clinical data (eg, cardiac function, New York Heart Association, and survival).

All patients were in a stable condition without preoperative respiratory or inotropic support. Standard CPB referred to a pump prime prepared to the current ''best practice'' protocols, which has an oxygen partial pressure that is relatively hyperoxic for a cyanotic patient. The hospital research ethics committee approved the present study, and parental informed consent was obtained for all patients. Treatment allocations, stratified by age (<6 months vs ≥6 months), were generated by computer in advance of starting the study, using block randomization with varying block sizes. The surgical team, with the exception of the perfusionists, were unaware of the treatment allocation. The preoperative characteristics of the 2 groups for the single- and double-ventricle patients are summarized in Table 1 and Table E1.

All operations were performed with CPB. The intraoperative anesthetic and operative techniques were standardized as previously reported.[12] Cold blood (4°C-6°C) St Thomas' no. I-based blood cardioplegic solution (4:1 dilution blood/St Thomas' no. I crystalloid cardioplegia) was used for myocardial preservation, with the following composition: 16 mM $MgCl_2$, 2 mM $CaCl_2$, 20 mM KCl, 147 mM NaCl, and 1.0 mM procaine HCl. Additional cardioplegia was administered after each 20 minutes of aortic crossclamping. Postoperatively, all the patients were admitted to the pediatric intensive care unit and were treated according to the unit protocols[12,13] by intensivists and pediatric cardiologists who were unaware of the treatment allocation.

CPB and Control of Oxygen Partial Pressure

The reoxygenation strategy when starting CPB was developed in our clinical perfusion science department.

Controlled reoxygenation CPB. The CPB circuit was set up and primed in accordance with the protocol,[5,13] usually with a red blood cell/albumin prime solution or, occasionally, a clear prime solution, depending on the patient's hemoglobin level. Just before the initiation of CPB, medical nitrogen was delivered to the gas exchange device (oxygenator) by way of a bacteriologic filter (0.2 μm) at a rate of 100 to 200 mL/min, and the prime was circulated at approximately 1000 mL/min. An in-line PaO_2 monitor was used to measure the PaO_2 of the prime. Using this technique, we were able to reduce the PaO_2 of the prime fluid to match that of the patient's own PaO_2 levels. Finally, before CPB was established, the prime PaO_2 was confirmed using a point-of-care blood gas analyzer, and the in-line PaO_2 monitor was calibrated. CPB was initiated in this relatively ''normoxic'' manner, and the PaO_2 levels of the arterialized blood were adjusted accordingly during CPB (Table 1).

Standard (hyperoxic) CPB group. Oxygen delivery was run at 100% to maintain the arterial oxygen saturation at >95% and PaO_2 levels of 150 to 200 mm Hg when starting CPB (Table 1 and Table E1).

Biomarkers of Organ Injury and Stress

The primary endpoints were the release of troponin I (TnI) (enzyme-linked immunosorbent assay; Access Immunoassay System, Beckman Instruments Inc, Fullerton, Calif) and 8-isoprostane (enzyme immunoassay; Cayman Chemicals, Ann Arbor, Mich) as measurements of myocardial cell damage and oxidative stress, and the release of markers of the whole body inflammatory response, including complement activation (C3α; BD OptEIA Human C3a ELISA; BD Biosciences, Franklin Lakes, NJ), interleukin (IL)-6, IL-8, IL-10, and microphage migration inhibitor factor (MIF) (enzyme-linked immunosorbent assay; Amersham Biosciences UK, Little Chalfont, United Kingdom), and stress response (cortisol; Access Immunoassay System, Beckman Coulter, Pasadena, Calif). Cerebral injury was assessed by the postoperative release of protein S100 (CanAg S100 EIA; CanAg Diagnostics AB, Goteborg, Sweden), and α-glutathione S-transferase (Biotrin High Sensitivity Alpha GST EIA Assay; Biotrin International, Dublin, Ireland) was used to assess hepatic cell damage.

Blood (2-3 mL) was collected preoperatively, at 10 and 30 minutes after starting CPB, and at 10 minutes and 4 and 24 hours after the cessation of CPB. This was immediately centrifuged at 4°C, at 4000 rpm for 15 minutes. The resulting plasma was then frozen in liquid nitrogen before storage at −80°C. A laboratory technician, who was unaware of the treatment allocation and clinical status of the patient, performed the assays.

The clinical outcomes (inotropic support, renal failure, intubation time, postoperative hospital stay, New York Heart Association class, and survival at follow-up) were also recorded. Myocardial function was assessed preoperatively, immediately postoperatively, and during follow-up for patients with a single ventricle using commercially available instruments (Vivid 7 imaging device, GE Healthcare, Little Chalfont, United Kingdom). All echocardiographic and Doppler data were obtained in digital format and stored on a workstation for offline analysis (EchoPAC; GE Vingmed Ultrasound AS, Horten, Norway). Ventricular function was defined as

TABLE 1. Preoperative characteristics, intra- and postoperative data in single-ventricle patients exposed to standard or controlled reoxygenation

Variable	Standard (hyperoxic) (n = 16)	Controlled reoxygenation (n = 16)	*P* value
Age (mo)	135 (55-206)	123 (68.3-169)	.5
Male sex	9 (57)	10 (62)	.7
Weight (kg)	16.1 (11.6-19.6)	13.9 (10.6-19.6)	.7
Preoperative saturation (%)	77.7 ± 7.1	81.0 ± 7.5	.2
Pathologic entity			.2
Pulmonary atresia	5 (31)	11 (69)	
Tricuspid atresia	7 (44)	2 (13)	
Double inlet left ventricle	2 (13)	1 (6)	
Ebstein anomaly	2 (13)	1 (6)	
Mitral stenosis	0	1 (6)	
Operation			.4
Glenn shunt	8 (50)	7 (44)	
TCPC	8 (50)	9 (56)	
PaO_2			
At start of CPB	167.8 ± 70.2	55.4 ± 13.7	<.001
At 5 min of CPB	169.6 ± 61.5	63.7 ± 20.5	<.001
At 10 min of CPB	171.8 ± 55.9	65.8 ± 18.2	<.001
At 30 min of CPB	155.6 ± 39.0	114.4 ± 29.7	.001
Immediately after CPB	173.2 ± 56.2	159.4 ± 41.1	.4
CPB time (min)	72.2 (43-136)	67.2 (34-178)	.9
Crossclamp time (min)	6.1 ± 14.1	11.6 ± 21.9	.4
30-d mortality	0	0	
Ventilation time (min)	11.5 (2-33)	21.2 (2-188)	.4
Dopamine support off CPB (μg/kg/min)	6.1 ± 4.7	5.3 ± 5.3	.6
Dopamine support at peak dose (μg/kg/min)	10.3 ± 6.3	9.8 ± 5.3	.8
Dopamine support duration (h)	62.6 ± 61.6	40.3 ± 62.8	.5
Length of hospital stay (d)	20.5 (7.7-28.5)	13.0 (6.0-17.0)	.7

Data presented as median (interquartile range), n (%), or mean ± standard deviation. *TCPC*, Total cavopulmonary connection; *PaO_2*, partial pressure of oxygen in arterial blood; *CPB*, cardiopulmonary bypass.

good or mildly impaired (ejection fraction >50%), moderately impaired (ejection fraction <50% but >30%), or severely impaired (ejection fraction <30%).[14]

Sample Size

The sample size was calculated from the results of previous similar studies performed at our institution.[11,12] With 1 preoperative and 5 postoperative measurements, a sample size of 15 to 20 per group would have >90% power to detect effect sizes of ≥0.5 for both markers at the 5% statistical significance level (2-tailed), assuming a correlation of 0.7 between the preoperative and postoperative values and among the postoperative measures.

Statistical Analysis

Continuous outcomes are expressed as the mean ± standard deviation if normally distributed or the geometric mean or median and interquartile range if skewed. Categorical data are presented as actual counts and percentages. Skewed measures were log-transformed to achieve normality, and the results were back transformed to the original scale. Biochemical markers measured at multiple points were analyzed using a mixed regression model. All the markers had a skewed distribution and were analyzed on the logarithmic scale. These analyses were adjusted for age, baseline response, pathologic findings, and the interaction between the pathologic entity and time and the treatment and time. An overall estimate, pooled over all measurement points, is reported. Effect sizes are reported as the mean differences (if normally distributed) or as ratios of geometric means (if skewed), with corresponding 95% confidence intervals (CIs) and *P* values. Survival was evaluated using the Kaplan-Meier method, and the log-rank test was used to compare the 2 groups.

RESULTS

The intraoperative and clinical outcomes for the single- and double-ventricle patients are listed in Table 1 and Table E1. No deaths occurred in this series ≤30 days postoperatively. Overall, no significant differences were found in terms of the perioperative clinical outcomes between the controlled reoxygenation CPB and standard (hyperoxic) CPB groups in either the single- or double-ventricle patients.

Two patients in the standard (hyperoxic) CPB group who underwent total cavopulmonary connection died within 1 year postoperatively of cardiac-related causes and 1 patient in the same group died 2 years after a Glenn procedure of bowel perforation and peritonitis. At a median follow-up of 7 years for patients with a single ventricle, survival was 100% in the controlled reoxygenation CPB group and 81% in the standard (hyperoxic) CPB group ($P = .03$; Figure E1). The double-ventricle patients had 100% survival with or without either intervention. Overall, more patients in the standard (hyperoxic) CPB group were in New York Heart Association class II and III (Figure 1, *A*) compared with the controlled reoxygenation CPB group (55.6% vs 22.5%). This difference was more prominent for single-ventricle patients (76.9% vs 23.1%

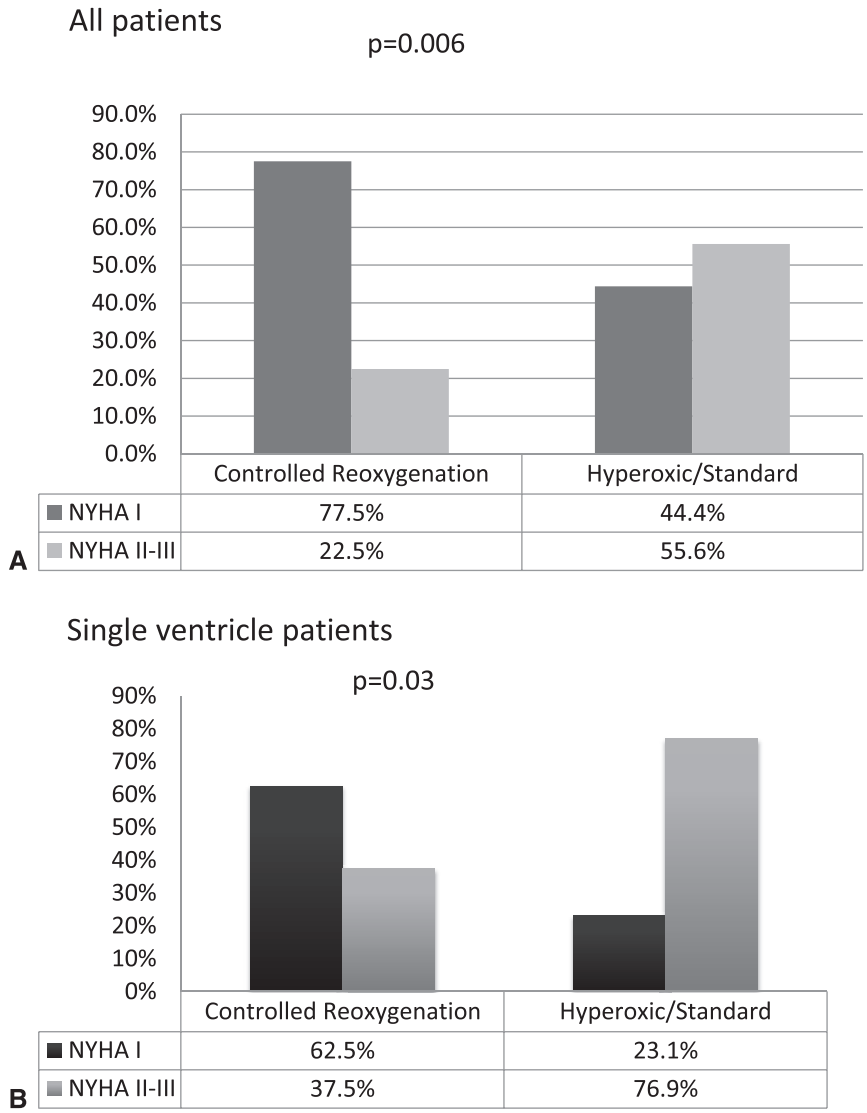

FIGURE 1. New York Heart Association (*NYHA*) class at follow-up (mean, 7 years) in (A) all patients or (B) patients with a single ventricle in the standard (hyperoxic) and controlled reoxygenation groups.

for standard and controlled reoxygenation CPB, respectively; Figure 1, *B*).

The preoperative and long-term (7 years) cardiac function classifications for patients with a single ventricle are shown in Figure 2.

Markers of Inflammation, Stress, and Organ Injury in Single-Ventricle Patients

Controlled reoxygenation CPB significantly decreased the inflammatory and stress response compared with standard (hyperoxic) CPB in patients undergoing single ventricle surgical correction (Figure 3). Furthermore, the overall release of ILs (IL-6, IL-8, IL10), C3α, MIF, and cortisol was significantly reduced by controlling the oxygen levels when starting CPB (Table E2).

At 10 minutes after starting CPB, the TnI levels were significantly greater than baseline in both groups (Figure 4, Table E2). The levels had peaked at 4 hours after surgery and remained high (compared with baseline) at 24 hours. Overall, the TnI levels were significantly lower in the controlled reoxygenation CPB group than in the standard (hyperoxic) CPB group (ratio, 0.58; 95% CI, 0.51-0.65; $P < .01$).

Controlled reoxygenation CPB significantly decreased the postoperative 8-isoprostane release compared with standard (hyperoxic) CPB (ratio, 0.76; 95% CI, 0.65-0.91; $P = .002$).

In both groups, an increase in serum protein S100 occurred soon (10 minutes) after the initiation of CPB and peaked at 10 minutes after withdrawing CPB. The release of protein S100 was significantly lower in the controlled reoxygenation CPB group than in the standard (hyperoxic) CPB group (ratio, 0.78; 95% CI, 0.66-0.91; $P = .002$; Figure 4, Table E2).

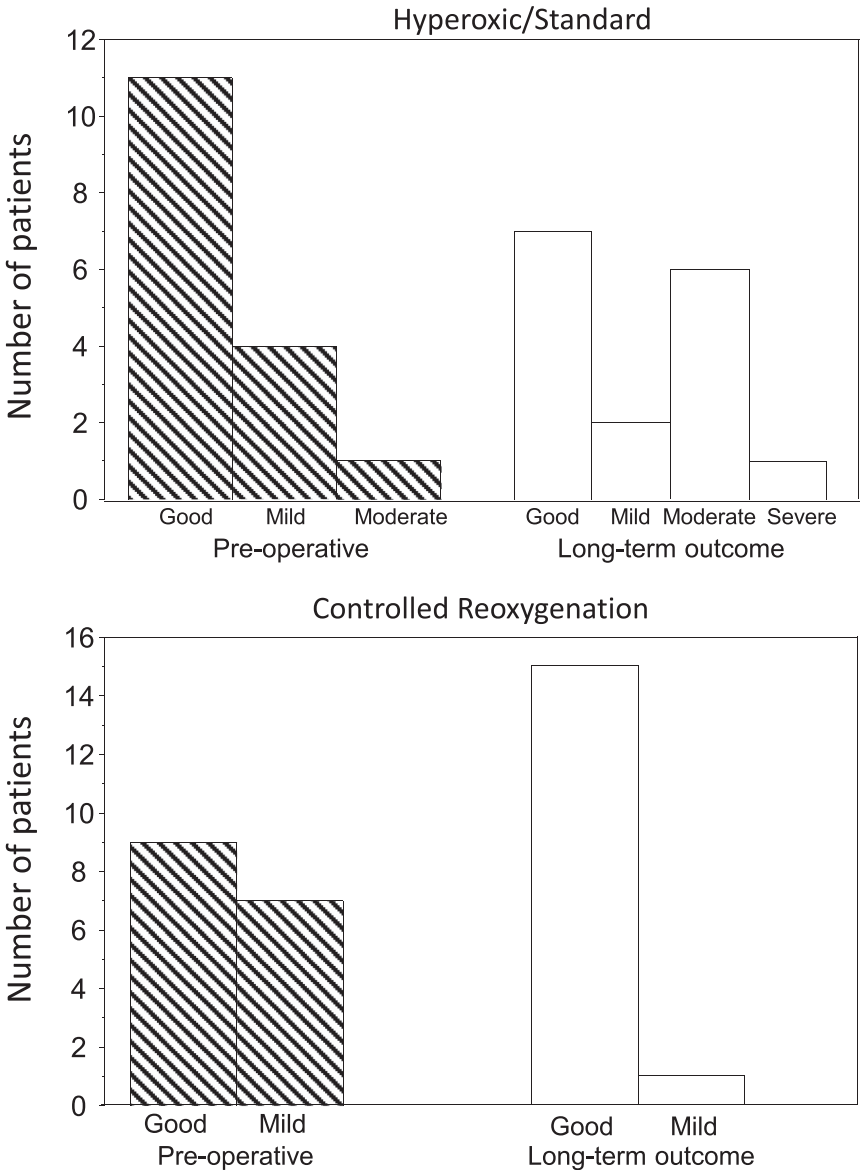

FIGURE 2. Cardiac function classification in single-ventricle patients in the standard (hyperoxic) and controlled reoxygenation groups. The change in classification between the preoperative and long-term outcomes was significantly ($P < .01$, Mann-Whitney U test for unpaired comparison) improved with controlled reoxygenation compared with standard (hyperoxic) reoxygenation.

α-Glutathione S-transferase peaked significantly at 4 hours after termination of CPB. Overall, a significant reduction occurred in serum α-glutathione S-transferase release in the controlled reoxygenation CPB group compared with that in the standard (hyperoxic) CPB group (ratio, 0.71; 95% CI, 0.60-0.85; $P < .01$; Figure 4, Table E2).

Markers of Inflammation, Stress, and Organ Injury in Double-Ventricle Patients

In patients with double ventricular anatomy undergoing surgical correction, controlling reoxygenation did not seem to significantly decrease the postoperative release of IL-6, IL-8, C3α, and MIF. However, a significant effect was found in reducing the stress response (cortisol) compared with using standard (hyperoxic) CPB (Figure E2, Table E3).

The TnI levels peaked at 10 minutes after cessation of CPB and started to decline from that point onward (Figure E3, Table E3). No statistically significant difference was found in the release of TnI between the controlled reoxygenation and standard (hyperoxic) CPB groups ($P = .31$).

Controlled reoxygenation CPB significantly decreased postoperative 8-isoprostane release compared with standard (hyperoxic) CPB (ratio, 0.52; 95% CI, 0.47-0.60; $P < .01$).

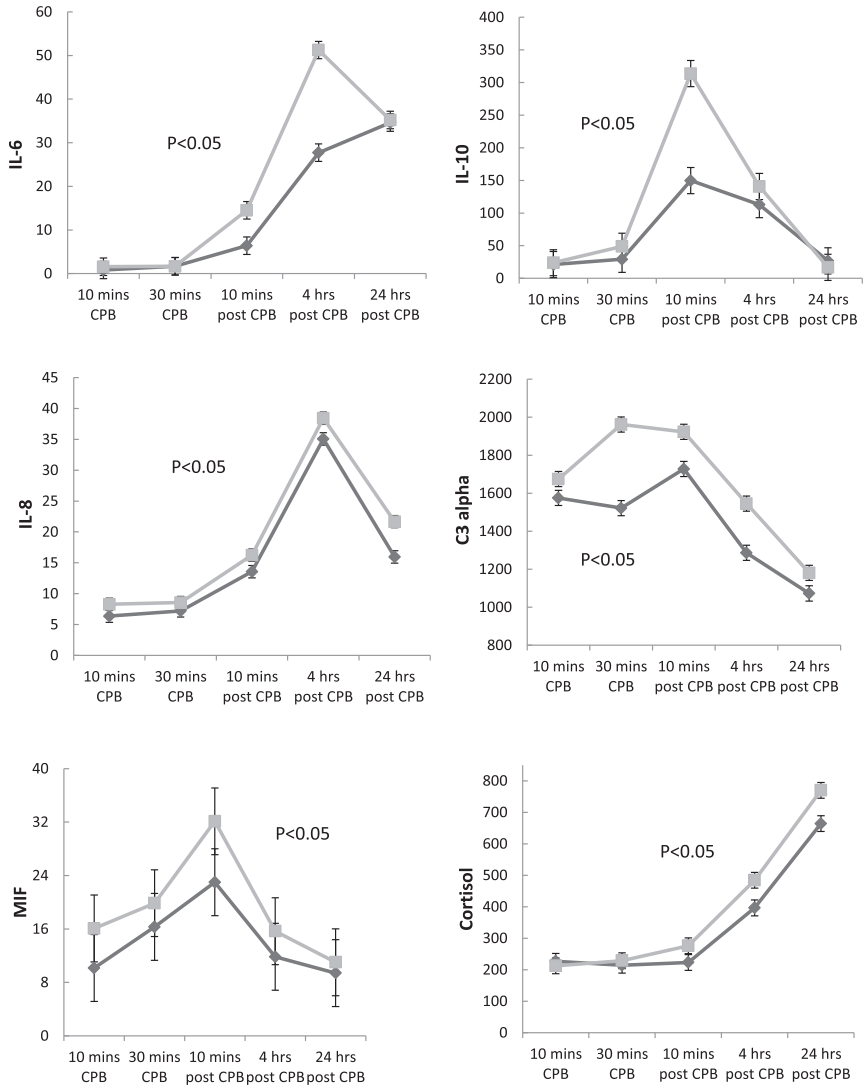

FIGURE 3. Time-related plasma changes in the geometric mean for interleukin (*IL*)-6, IL-10, IL-8, complement activation (*C3α*), macrophage migration inhibitor factor (*MIF*), and cortisol in patients with a single ventricle in the standard (hyperoxic) (*squares*) and controlled reoxygenation (*diamonds*) cardiopulmonary bypass (*CPB*) groups. Unit of concentration, ng/mL.

The release of protein S100 in the controlled reoxygenation group was significantly lower than in the standard (hyperoxic) CPB group (ratio, 0.83; 95% CI, 0.74-0.93; $P < .01$; Figure E3, Table E3). The α-glutathione S-transferase level peaked significantly at 4 hours after termination of CPB. Overall, a significant reduction was seen in serum α-glutathione S-transferase release in the controlled reoxygenation CPB group compared with the standard (hyperoxic) CPB group (ratio, 0.87; 95% CI, 0.78-0.99; $P < .01$; Figure E3, Table E3).

DISCUSSION

The present study reports several novel findings relating to the protection of cyanotic patients with a single or double ventricle during corrective surgery. Controlled reoxygenation (low PaO_2, normoxic to patients) when starting CPB in cyanotic patients with a single ventricle reduced the markers of organ injury, systemic inflammation, and stress compared with standard reoxygenation (physiologic PaO_2, hyperoxic to patients). However, the protective efficacy of this intervention was markedly reduced in patients with a double ventricle. The mechanism of action of controlled reoxygenation CPB in these cyanotic patients is likely to be the gradual reintroduction of oxygen into chronically hypoxic organs (eg, the heart[5]). However, the main finding of our study was that controlled reoxygenation is only effective at reducing the markers of inflammation and cardiac injury in those with a single ventricle. Although patients with a single or double ventricle are cyanotic, key differences exist that can explain why this

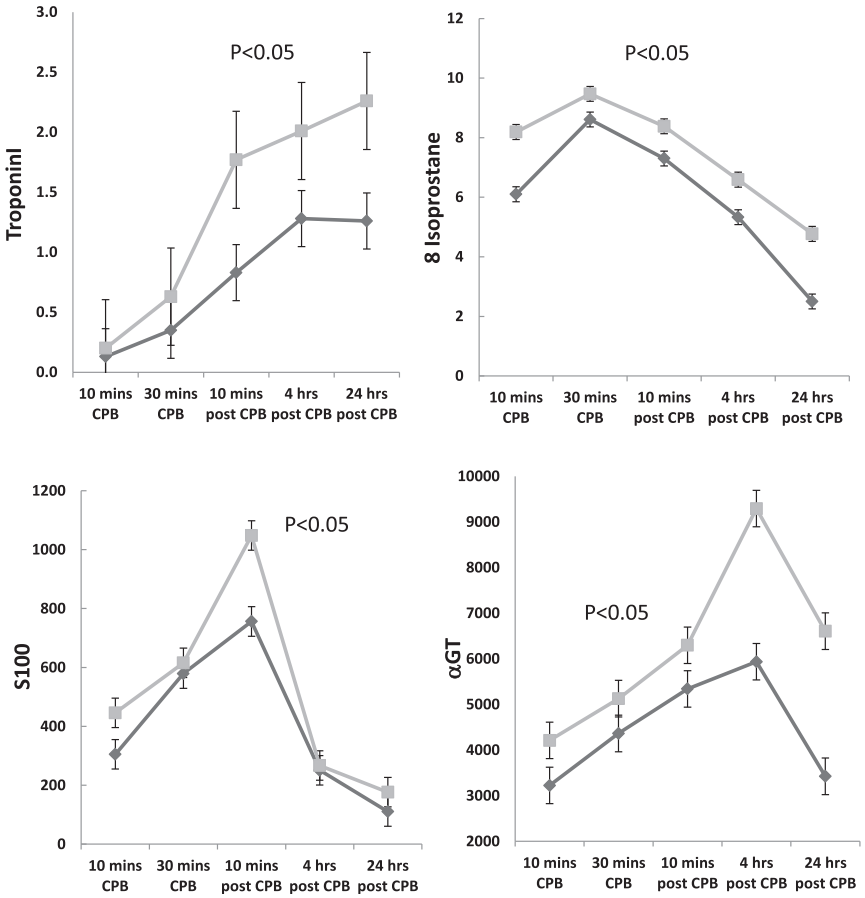

FIGURE 4. Time-related plasma changes in the geometric mean for troponin I, 8-isoprostane, protein S100, and α-glutathione S-transferase in patients with a single ventricle in the standard (hyperoxic) (*squares*) and controlled reoxygenation (*diamonds*) cardiopulmonary bypass (*CPB*) groups. Unit of concentration, ng/mL. *α-GT*, α-Glutathione S-transferase.

intervention was more effective in single-ventricle patients. Cyanosis in single-ventricle patients remains at a relatively constant level, rendering the heart (and other organs) chronically hypoxic. In contrast, the degree of cyanosis in patients with a double ventricle tends to be lower and to fluctuate. It is likely, therefore, that single-ventricle patients will sustain more reoxygenation injury when receiving standard (hyperoxic) CPB. A greater degree of organ reoxygenation injury in single-ventricle patients will trigger relatively more oxidative stress and inflammatory response.[15]

Controlled Reoxygenation and Biomarkers of Inflammation and Oxidative Stress

Our suggestion that single-ventricle patients will sustain significant reoxygenation injury is supported by a recent study showing that immediately after CPB the levels of markers of oxidative stress are increased[16] and that oxidative stress precedes the peak systemic inflammatory response.[17] The 8-isoprostane levels (oxidative stress) will peak during CPB, and most inflammatory markers (ILs and complement C3α) will hardly change during this period (Figures 3 and 4, Figures E2 and E3). The C3α levels were particularly high in single-ventricle patients (Figure 3) and have been previously associated with effusion and edema after CPB in pediatric patients.[18,19]

Single-ventricle anatomy is a key risk factor for operative mortality.[3] In contrast, the role of CPB-induced systemic inflammatory response in postoperative morbidity appears to be limited in infants undergoing low-to-moderate complexity cardiac surgery.[20]

Despite the link between the inflammatory response and postoperative complications, attempts at reducing this response (eg, using steroids) during pediatric cardiac surgery have not appeared to produce significant effects on postoperative recovery.[21] The identification of other mediators of inflammation, including MIF, might provide another strategy in the treatment of postoperative inflammation in heart surgery patients.[22-24] Work has shown that oxidative stress induces secretion of MIF from the cardiomyocytes themselves.[25] Our data have strongly indicated that increased circulating levels of MIF are

associated with high levels of TnI and 8-isoprostane (Figures 3 and 4). The finding that MIF was reduced by controlled reoxygenation only in single-ventricle patients is consistent with the intervention reducing cardiac injury (Figures 3 and 4). Reducing the levels of MIF is important, because reports have shown that it depresses human myocardial contractile function and impairs mitochondrial respiration.[26] The same trend was not observed in patients with a double ventricle, in whom controlling reoxygenation did not significantly influence the release of MIF and TnI.

Controlled Reoxygenation and Organ Injury

Surgical correction can trigger a significant systemic inflammatory and stress response with the postoperative increase of ILs, complement C3α, and cortisol. A devastating consequence of the systemic inflammatory response is the development of remote organ injury and multiorgan dysfunction syndrome.[27] This can lead to pulmonary, hepatic, renal, gastrointestinal, myocardial, and cerebral dysfunction.[28] Several studies have shown a significant positive correlation between the magnitude of the inflammatory response as measured by the serum concentrations of IL-6 and IL-8 after CPB and the degree of medical intervention after pediatric heart surgery, particularly in patients with single-ventricle physiology.[19] This group of patients represents a particularly high-risk population in terms of early- and long-term myocardial and cerebral dysfunction. Therefore, strategies aimed at reducing the intraoperative inflammatory and stress response with the associated multiple organ damage are warranted.[1] However, the relevance of these biomarkers in terms of clinical benefit can only be established using a much larger sample size with clinical primary endpoints. Our larger randomized controlled trial, which has just completed recruitment of 220 patients, might provide some of the answers.

CONCLUSIONS

Our study has provided direct evidence that, in single-ventricle patients, controlling reoxygenation will reduce the blood markers of inflammation, stress, and markers of myocardial, cerebral, and hepatic injury.

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References

1. Goldberg C. Neurocognitive outcomes for children with functional single ventricle malformations. *Pediatr Cardiol*. 2007;28:443-7.
2. Butler J, Pathi VL, Paton RD, Logan RW, MacArthur KJ, Jamieson MP, et al. Acute-phase responses to cardiopulmonary bypass in children weighing less than 10 kilograms. *Ann Thorac Surg*. 1996;62:538-42.
3. Curi-Curi PJ, Cervantes-Salazar J, Calderon-Colmenero J, Garcia-Montes JA, Ramirez S. [Operative results in cardiovascular surgery of the neonate]. *Rev Invest Clin*. 2012;64:199-206.
4. Morris MC, Ittenbach RF, Godinez RI, Portnoy JD, Tabbutt S, Hanna BD, et al. Risk factors for mortality in 137 pediatric cardiac intensive care unit patients managed with extracorporeal membrane oxygenation. *Crit Care Med*. 2004;32:1061-9.
5. Modi P, Imura H, Caputo M, Pawade A, Parry A, Angelini GD, et al. Cardiopulmonary bypass-induced myocardial reoxygenation injury in pediatric patients with cyanosis. *J Thorac Cardiovasc Surg*. 2002;124:1035-6.
6. del Nido PJ, Benson LN, Mickle DA, Kielmanowicz S, Coles JG, Wilson GJ. Impaired left ventricular postischemic function and metabolism in chronic right ventricular hypertrophy. *Circulation*. 1987;76:V168-73.
7. del Nido PJ, Mickle DA, Wilson GJ, Benson LN, Weisel RD, Coles JG, et al. Inadequate myocardial protection with cold cardioplegic arrest during repair of tetralogy of Fallot. *J Thorac Cardiovasc Surg*. 1988;95:223-9.
8. Ihnken K, Morita K, Buckberg GD, Ignarro LJ, Beyersdorf F. Reduction of reoxygenation injury and nitric oxide production in the cyanotic immature heart by controlling pO_2. *Eur J Cardiothorac Surg*. 1995;9:410-8.
9. Ihnken K, Morita K, Buckberg GD. Delayed cardioplegic reoxygenation reduces reoxygenation injury in cyanotic immature hearts. *Ann Thorac Surg*. 1998;66:177-82.
10. Ihnken K, Winkler A, Schlensak C, Sarai K, Neidhart G, Unkelbach U, et al. Normoxic cardiopulmonary bypass reduces oxidative myocardial damage and nitric oxide during cardiac operations in the adult. *J Thorac Cardiovasc Surg*. 1998;116:327-34.
11. Caputo M, Mokhtari A, Rogers CA, Panayiotou N, Chen Q, Ghorbel MT, et al. The effects of normoxic versus hyperoxic cardiopulmonary bypass on oxidative stress and inflammatory response in cyanotic pediatric patients undergoing open cardiac surgery: a randomized controlled trial. *J Thorac Cardiovasc Surg*. 2009;138:206-14.
12. Modi P, Suleiman MS, Reeves B, Pawade A, Parry AJ, Angelini GD, et al. Myocardial metabolic changes during pediatric cardiac surgery: a randomized study of 3 cardioplegic techniques. *J Thorac Cardiovasc Surg*. 2004;128:67-75.
13. Imura H, Caputo M, Parry A, Pawade A, Angelini GD, Suleiman MS. Age-dependent and hypoxia-related differences in myocardial protection during pediatric open heart surgery. *Circulation*. 2001;103:1551-6.
14. Bruns LA, Chrisant MK, Lamour JM, Shaddy RE, Pahl E, Blume ED, et al. Carvedilol as therapy in pediatric heart failure: an initial multicenter experience. *J Pediatr*. 2001;138:505-11.
15. Suleiman MS, Zacharowski K, Angelini GD. Inflammatory response and cardioprotection during open-heart surgery: the importance of anaesthetics. *Br J Pharmacol*. 2008;153:21-33.
16. Albers E, Donahue BS, Milne G, Saville BR, Wang W, Bichell D, et al. Perioperative plasma f(2)-isoprostane levels correlate with markers of impaired ventilation in infants with single-ventricle physiology undergoing stage 2 surgical palliation on the cardiopulmonary bypass. *Pediatr Cardiol*. 2012;33:562-8.
17. Christen S, Finckh B, Lykkesfeldt J, Gessler P, Frese-Schaper M, Nielsen P, et al. Oxidative stress precedes peak systemic inflammatory response in pediatric patients undergoing cardiopulmonary bypass operation. *Free Radic Biol Med*. 2005;38:1323-32.
18. Bocsi J, Hambsch J, Osmancik P, Schneider P, Valet G, Tarnok A. Preoperative prediction of pediatric patients with effusions and edema following cardiopulmonary bypass surgery by serological and routine laboratory data. *Crit Care*. 2002;6:226-33.
19. Madhok AB, Ojamaa K, Haridas V, Parnell VA, Pahwa S, Chowdhury D. Cytokine response in children undergoing surgery for congenital heart disease. *Pediatr Cardiol*. 2006;27:408-13.
20. Allan CK, Newburger JW, McGrath E, Elder J, Psoinos C, Laussen PC, et al. The relationship between inflammatory activation and clinical outcome after infant cardiopulmonary bypass. *Anesth Analg*. 2010;111:1244-51.
21. Gessler P, Hohl V, Carrel T, Pfenninger J, Schmid ER, Baenziger O, et al. Administration of steroids in pediatric cardiac surgery: impact on clinical outcome and systemic inflammatory response. *Pediatr Cardiol*. 2005;26:595-600.
22. de Mendonca-Filho HT, Gomes RV, de Almeida Campos LA, Tura B, Nunes EM, Gomes R, et al. Circulating levels of macrophage migration inhibitory factor are associated with mild pulmonary dysfunction after cardiopulmonary bypass. *Shock*. 2004;22:533-7.
23. Bozza FA, Gomes RN, Japiassu AM, Soares M, Castro-Faria-Neto HC, Bozza PT, et al. Macrophage migration inhibitory factor levels correlate with fatal outcome in sepsis. *Shock*. 2004;22:309-13.

24. Lin X, Yang H, Sakuragi T, Hu M, Mantell LL, Hayashi S, et al. Alpha-chemokine receptor blockade reduces high mobility group box 1 protein-induced lung inflammation and injury and improves survival in sepsis. *Am J Physiol Lung Cell Mol Physiol*. 2005;289:L583-90.
25. Fukuzawa J, Nishihira J, Hasebe N, Haneda T, Osaki J, Saito T, et al. Contribution of macrophage migration inhibitory factor to extracellular signal-regulated kinase activation by oxidative stress in cardiomyocytes. *J Biol Chem*. 2002;277:24889-95.
26. Preau S, Montaigne D, Modine T, Fayad G, Koussa M, Tardivel M, et al. Macrophage migration inhibitory factor induces contractile and mitochondria dysfunction by altering cytoskeleton network in the human heart*. *Crit Care Med*. 2013;41:e125-33.
27. Grace PA, Mathie RT. *Ischaemia-Reperfusion Injury*. Oxford: Blackwell Science; 1999.
28. Ware LB, Matthay MA. The acute respiratory distress syndrome. *N Engl J Med*. 2000;342:1334-49.

Discussion

Dr David P. Bichell *(Nashville, Tenn).* Thank you for very thought-provoking and well-presented data. Your group's work has helped frame the importance of hyperoxic injury from the initiation of cardiopulmonary bypass, especially in cyanotic infants, and now you very elegantly go further to show that the injury is greatest for the single-ventricle cyanotic patients.

Other investigators have studied post-bypass hyperoxia-induced stress injury and then have focused on pharmacologic agents or other interventions to try to stamp out the fire already lit by cardiopulmonary bypass and its inflammatory sequelae. But your work points to a very appealing, practical, and more preemptive approach of getting at the cause of it, and your controlled reoxygenation strategy is appealing, especially pertinent to the single-ventricle population, vulnerable in so many ways and undergoing multiple insults on bypass, the consequences of which compound.

I have a couple of questions.

The first is methodologic. In your 7-year follow-up data, the single-ventricle patients, enrolled during the time of their Glenn shunt, no doubt also underwent a Fontan procedure within the study period. So that is 2 CPB runs. I just wonder how you treated that with respect to the study design. In other words, if they were randomized for hyperoxic versus controlled CPB at the first operation, were those patients tracked and treated similarly at the second insult of CPB? I am interested in how that fits in your model and how that might affect the long-term data which, of course, would then be influenced by multiple other factors.

Dr Caputo. If, for example, a patient undergoing a Glenn shunt was randomized the first time, we could not recruit the same patient for the total cavopulmonary connection. So when they came to the Fontan, they were not randomized anymore. But we did use the same strategy that was used for the first operation.

In a way, our study does really specifically consider the early outcomes and biochemical markers of damage. Regarding the follow-up, I do not think we have a significant number to have a definite answer on the follow-up. So the outcomes of the follow-up are more observational. We just wanted to present for the completeness of the data, but we are not really saying that with these numbers we randomized, we could have a definite answer on the follow-up in terms of survival and ventricular function.

Dr Bichell. It is an interesting striking difference in the follow-up.

Dr Caputo. There is, yes.

Dr Bichell. It will be interesting to see more numbers for that.

Dr Caputo. Absolutely.

Dr Bichell. You divided your population into the single-ventricle and biventricular patients, a heterogeneous mix of each, and you nicely showed that a difference exists between the 2. Also, in the report, you speculated that the difference between these 2 might be something to do with the chronicity and depth of the cyanosis, with each respective group looking different, and, therefore, the shock of hyperoxia looking different in 1 group versus another.

But I wonder if there is not a more fundamental difference to consider and perhaps consideration of dividing the groups differently to be inclusive of the physiology that might drive this. By that I mean, it is known that the lung is responsible for an especially large portion of the reactivity of hyperoxic injury, and it seems to me that the single-ventricle patients versus the biventricular patients in general have parallel versus series circulation. We are talking about an insult that occurs at the initiation of CPB. In the parallel circulation patient, for example, with shunt-dependent pulmonary blood flow, the initiation of CPB results in distension of the pulmonary arterial tree with hyperoxic blood, a shock, if you will, to the hypoxic initial state. But for a septated, biventricular heart in series circulation, such as a patient with tetralogy of Fallot, it is the opposite. The pulmonary tree is actually emptied at the initiation of CPB. Also, if there is hyperoxic injury, it is potentially more directed to organs other than the lungs.

So I wonder if dividing the patients by series versus parallel circulation, each group of which would contain some single and some biventricular patients, you might further refine this effect and/or explain it physiologically. That is a long way of asking, have you considered this and were there patients in your study who were, for example, a patient with tetralogy of Fallot with major aortopulmonary collateral arteries who essentially had systemic pulmonary circulation whose data can be analyzed to see whether that is more of a physiologic explanation for the difference you are seeing?

Dr Caputo. This is a really good suggestion. We have not analyzed the data in that respect. Probably now that we have finished the trial with >200 patients recruited, we should be able to, because we might have enough numbers to subdivide into those categories.

In this subgroup, some patients had a previous shunt and possibly major aortopulmonary collateral arteries, but there were not that many, so, again, to subdivide it into that group would probably dilute the groups too much. But it is definitely something that we should do with the larger cohort of patients.

In terms of why the single ventricle benefits more, several explanations are possible. I think, as you suggested, there are definitely patients with a double ventricle, such as, possibly, those with transposition of the great arteries or very cyanotic tetralogy with a Blalock-Taussig shunt, who could benefit as well. However, perhaps, the ischemia reperfusion they undergo on top of the oxygenation might have masked the effects of the benefits of the oxygenation, because in single-ventricle patients, you either do

not crossclamp or the crossclamp time is very short. Thus, probably the beneficial effect of controlling the reoxygenation is not as evident in the biventricular repair where you do have to crossclamp.

Dr Bichell. Well, this is very thought-provoking study and one that I think can be integrated into a real practice change. I know I had not before considered anything other than the dogmatic approach of hyperoxic CPB, and you have given me pause to think about this. I think this is an elegant method to connect data to a change of practice.

Dr Caputo. Yes, thanks. Also, the good thing is that it is very simple technique in a way, because it literally involves flushing the CPB prime with nitric and it does not really affect the surgery, the operation itself, so it is a quite simple technique.

Dr Meena Nathan *(Boston, MA).* Are you planning to study the neurologic outcomes of these patients? Do you have any early data on that?

Dr Caputo. We just finished recruiting 230 patients, and we followed them with Bayley's and other neurocognitive tests for 6 months to $\leq$2 years, so I think the data will be available next year, yes. That is our primary target, studying the neurologic outcomes.

Dr Duke E. Cameron *(Baltimore, Md).* I enjoyed the study very much as well. A quick question about your protocol. If I read the slides right, on your controlled reoxygenation, you were hypoxic at the institution of CPB but you allowed the oxygen partial pressure to increase.

The reason I am asking this is that controlled reoxygenation injury is worse in ischemic tissues than in those normally perfused, and I am asking what was the oxygen partial pressure for these patients just before the crossclamp was removed? Because I was not clear of the rationale of lowering it when you institute CPB, but letting it increase again and be hyperoxic just before reperfusing the heart, which would be the organ most at risk of this kind of injury.

Dr Caputo. That is a very good observation. We based our strategy on our previous data. We analyzed the release of, for example, troponin I in cyanotic patients, and it appeared that it was the initial experience of the high oxygen during CPB that created the damage. We saw as early as at 10 minutes of CPB, an increase in the blood level of troponin I. So in the single ventricle, most of these patients did not have the crossclamp, so that was a very homogeneous group. That is why I think it was the best possible group to study this entity. In the double ventricle, I have not specifically concentrated on that. So, you are asking what was the oxygen partial pressure when we crossclamp, so before crossclamping?

Dr Cameron. Just before you came off.

Dr Caputo. We have not studied that. It is possibly a good suggestion, and we should have the data to analyze.

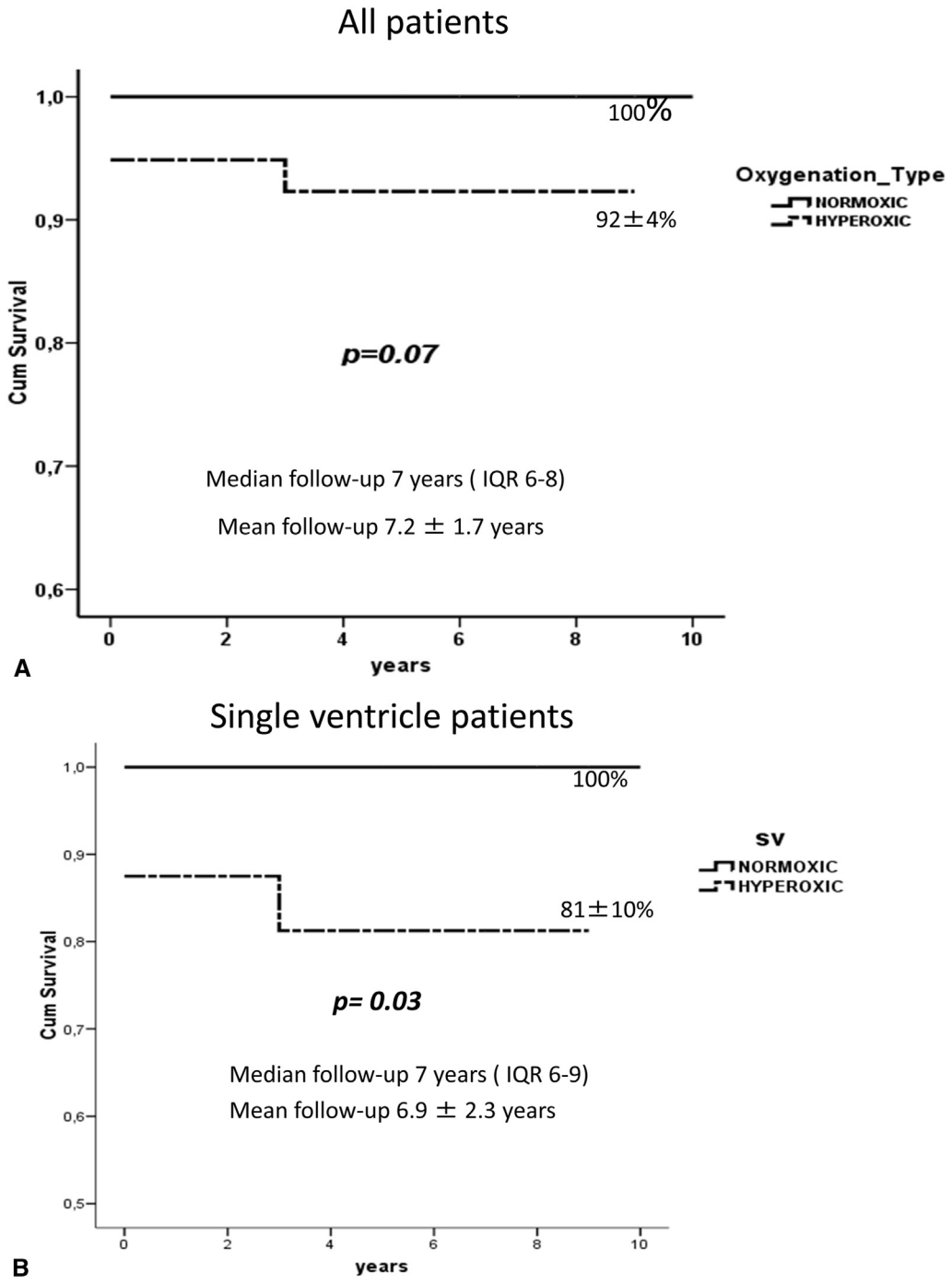

FIGURE E1. Survival at follow-up (median, 7 years) in (A) all patients and (B) patients with a single ventricle (*SV*) in the standard (hyperoxic) and controlled reoxygenation groups. *Cum*, Cumulative; *IQR*, interquartile range.

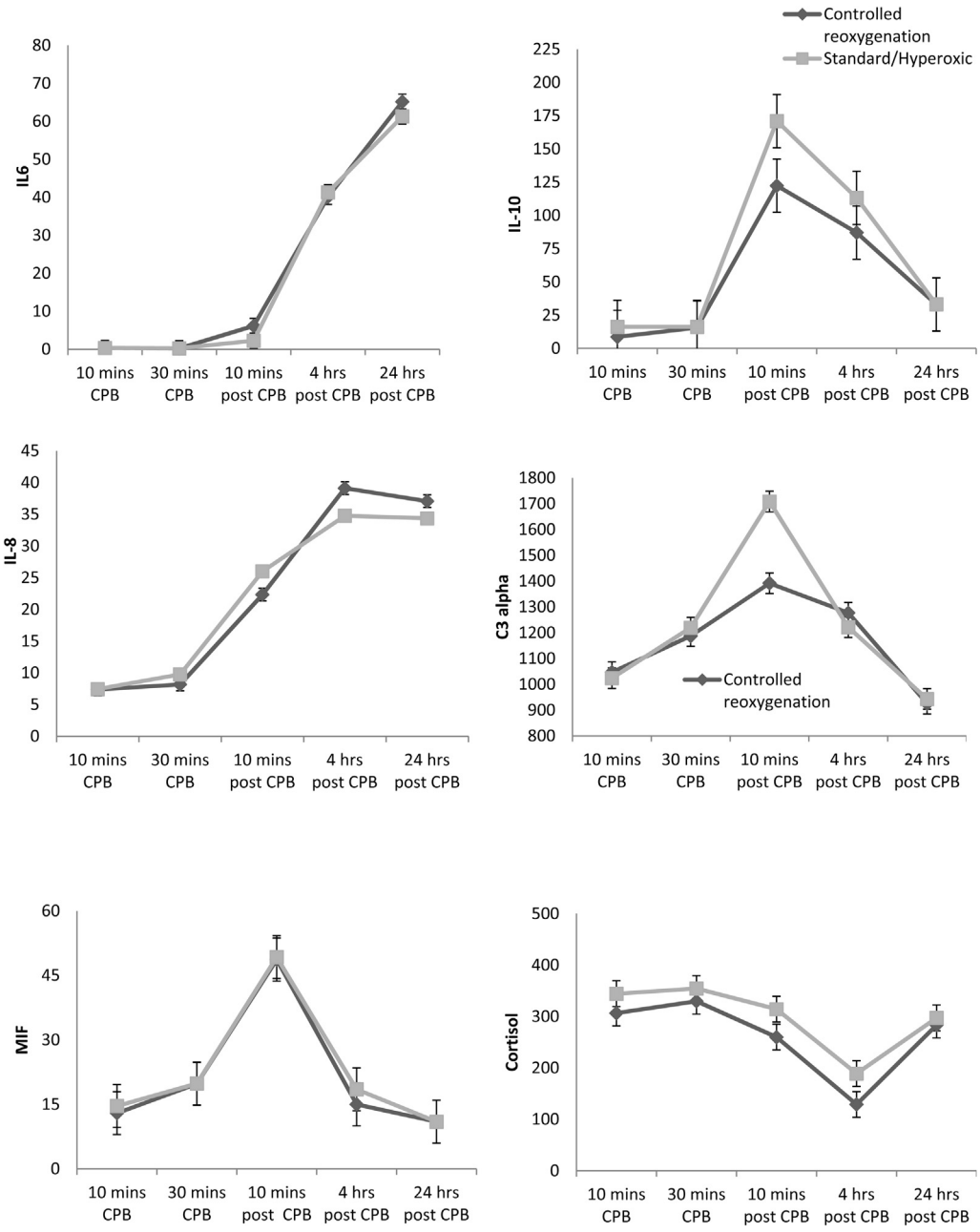

FIGURE E2. Time-related plasma changes in the geometric mean for interleukin (*IL*)-6, IL-10, IL-8, complement activation (*C3α*), macrophage migration inhibitor factor (*MIF*), and cortisol in patients with a double ventricle in the standard (hyperoxic) (*squares*) and controlled reoxygenation (*diamonds*) cardiopulmonary bypass (*CPB*) groups. Unit of concentration, ng/mL.

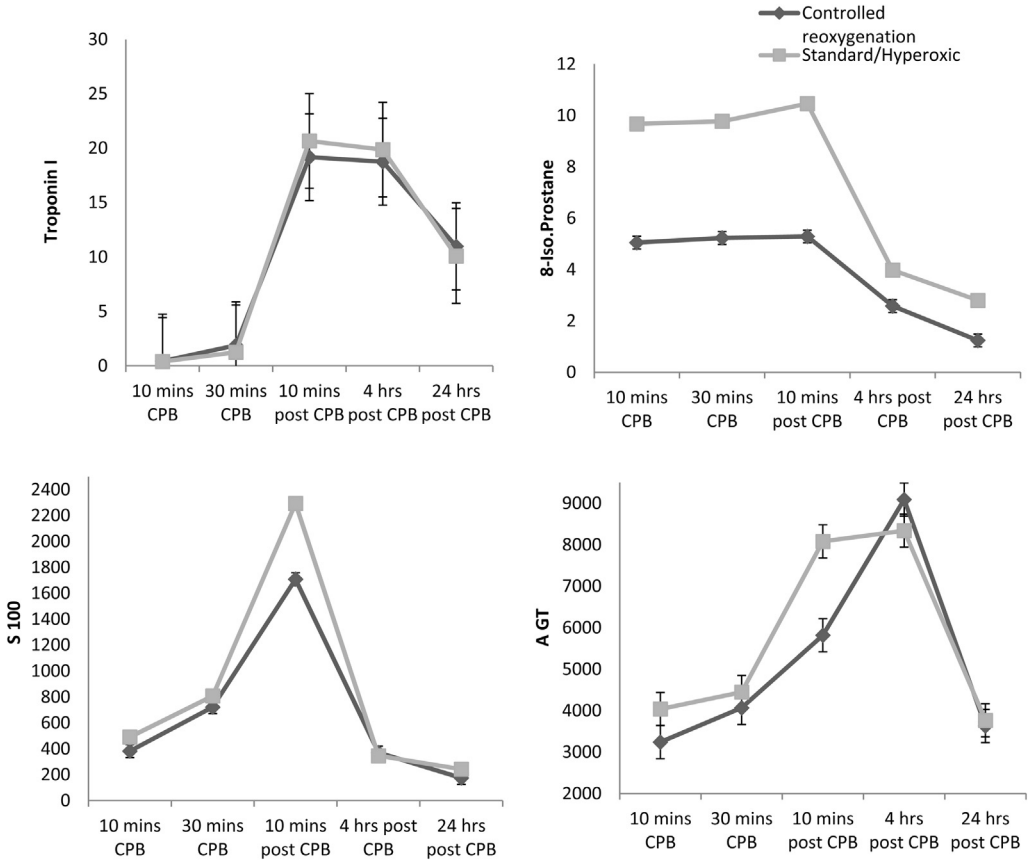

FIGURE E3. Time-related plasma changes in the geometric mean for troponin I, 8-isoprostane, protein S100, and α-glutathione S-transferase in patients with a double ventricle in the standard (hyperoxic) (*squares*) and controlled reoxygenation (*diamonds*) cardiopulmonary bypass (*CPB*) groups. Unit of concentration, ng/mL.

TABLE E1. Preoperative characteristics and intraoperative and postoperative data in cyanotic double-ventricle patients exposed to standard or controlled reoxygenation

Variable	Standard (n = 23)	Controlled reoxygenation (n = 24)	*P* value
Age (d)	223 (74-434)	217.5 (29-550)	.8
Male sex	13 (56)	16 (66)	.5
Weight (kg)	7.6 (4.7-9.8)	7.6 (3.6-9.3)	.4
Preoperative saturation (%)	81.4 ± 5.3	78.0 ± 10.1	.2
Pathologic entity			.1
Tetralogy of Fallot (complete repair)	16 (70)	15 (62)	
TGA (arterial switch operation)	5 (22)	7 (29)	
Other (aortic or mitral valve procedure)	2 (9)	2 (8)	
PaO_2			
At start of CPB	165.0 ± 61.1	51.7 ± 13.2	<.001
At 5 min of CPB	231.4 ± 60.1	66.0 ± 46.1	<.001
At 10 min of CPB	216.2 ± 56.7	79.0 ± 43.5	<.001
At 30 min of CPB	184.2 ± 34.0	116.3 ± 28.8	<.001
Immediately after CPB	171.4 ± 47.9	163.1 ± 45.6	.6
CPB time (min)	105.4 (60-210)	92.6 (50-147)	.9
Crossclamp time (min)	62.0 ± 25.7	54.7 ± 22.9	.1
30-d mortality	0	0	
Ventilation time (min)	38.8 (18-287)	33.6 (6-191)	.8
Dopamine support off CPB (μg/kg/min)	7.1 ± 4.5	7.2 ± 3.0	.9
Dopamine support peak dose (μg/kg/min)	10.1 ± 4.3	12.3 ± 4.6	.1
Dopamine support duration (h)	51.6 ± 58.6	53.3 ± 47.3	.9
Length of hospital stay (d)	7.0 (6.0-10.0)	7.5 (5.2-11.7)	.3

Data presented as median (interquartile range), n (%), or mean ± standard deviation. *TGA*, Transposition of the great arteries; *PaO_2*, partial pressure of oxygen in arterial blood; *CPB*, cardiopulmonary bypass.

TABLE E2. Biochemical marker release in patients with single-ventricle anatomy

Variable	Measurement point	Geometric mean* Controlled reoxygenation	Geometric mean* Standard (hyperoxic)	Ratio	95% CI	*P* value
Troponin I						
	Preoperatively					
	10 min on CPB	0.13	0.20	0.66		
	30 min on CPB	0.35	0.63	0.55		
	10 min off CPB	0.83	1.77	0.47		
	4 h after CPB	1.28	2.01	0.65		
	24 h after CPB	1.26	2.26	0.56		
	Test for interaction between treatment and time					.465
	Treatment effect, pooled over all points			0.58	0.51-0.65	<.01
8-Isoprostane						
	Preoperatively					
	10 min on CPB	6.10	8.19	0.74		
	30 min on CPB	8.61	9.47	0.91		
	10 min off CPB	7.30	8.38	0.87		
	4 h after CPB	5.33	6.59	0.81		
	24 h after CPB	2.50	4.77	0.52		
	Test for interaction between treatment and time					.45
	Treatment effect, pooled over all points			0.76	0.65-0.91	.002
IL-6						
	Preoperatively					
	10 min on CPB	0.82	1.57	0.51		
	30 min on CPB	1.70	1.66	1.02		
	10 min off CPB	6.40	14.53	0.45		
	4 h after CPB	27.73	51.26	0.54		
	24 h after CPB	34.63	35.21	0.98		
	Test for interaction between treatment and time					.39
	Treatment effect, pooled over all points			0.66	0.46-0.93	.02
IL-8						
	Preoperatively					
	10 min on CPB	6.36	8.28	0.76		
	30 min on CPB	7.21	8.56	0.85		
	10 min off CPB	13.57	16.24	0.83		
	4 h after CPB	35.07	38.42	0.91		
	24 h after CPB	15.96	21.67	0.72		
	Test for interaction between treatment and time					.9
	Treatment effect, pooled over all points			0.71	0.78-0.87	<.01
IL-10						
	Preoperatively					
	10 min on CPB	21.04	23.89	0.87		
	30 min on CPB	29.25	49.05	0.60		
	10 min off CPB	149.80	313.64	0.48		
	4 h after CPB	112.96	140.74	0.79		
	24 h after CPB	26.72	16.77	1.62		
	Test for interaction between treatment and time					.1
	Treatment effect, pooled over all points			0.78	0.58-1.05	.1
C3α						
	Preoperatively					
	10 min on CPB	1575.25	1674.40	0.95		
	30 min on CPB	1521.66	1961.73	0.79		
	10 min off CPB	1727.88	1923.11	0.91		
	4 h after CPB	1286.28	1545.58	0.83		
	24 h after CPB	1072.66	1181.17	0.91		
	Test for interaction between treatment and time					.52
	Treatment effect, pooled over all points			0.87	0.81-0.93	<.01

(Continued)

TABLE E2. Continued

Variable	Measurement point	Geometric mean*		Ratio	95% CI	*P* value
		Controlled reoxygenation	Standard (hyperoxic)			
Cortisol						
	Preoperatively					
	10 min on CPB	227.01	212.75	1.07		
	30 min on CPB	214.15	228.80	0.93		
	10 min off CPB	223.34	276.39	0.81		
	4 h after CPB	396.57	484.54	0.81		
	24 h after CPB	664.05	770.05	0.85		
	Test for interaction between treatment and time					.53
	Treatment effect, pooled over all points			0.91	0.83-0.99	.04
S100						
	Preoperatively					
	10 min on CPB	304.91	445.95	0.68		
	30 min on CPB	579.07	615.59	0.93		
	10 min off CPB	756.09	1047.88	0.72		
	4 h after CPB	250.73	266.72	0.93		
	24 h after CPB	110.34	176.45	0.62		
	Test for interaction between treatment and time					.1
	Treatment effect, pooled over all points			0.78	0.66-0.91	.002
α-GT						
	Preoperatively					
	10 min on CPB	3223.37	4210.38	0.78		
	30 min on CPB	4362.86	5125.54	0.85		
	10 min off CPB	5338.99	6296.52	0.85		
	4 h after CPB	5935.48	9290.56	0.63		
	24 h after CPB	3423.88	6604.01	0.51		
	Test for interaction between treatment and time					.52
	Treatment effect, pooled over all points			0.71	0.60-0.85	<.01
MIF						
	Preoperatively					
	10 min on CPB	10.16	16.08	0.63		
	30 min on CPB	16.31	19.86	0.81		
	10 min off CPB	23.00	32.12	0.71		
	4 h after CPB	11.84	15.67	0.76		
	24 h after CPB	9.40	11.02	0.85		
	Test for interaction between treatment and time					.44
	Treatment effect, pooled over all points			0.74	0.69-0.79	<.01

CI, Confidence interval; *CPB*, cardiopulmonary bypass; *IL*, interleukin; *C3α*, complementary activation; *α-GT*, α-glutathione S-transferase; *MIF*, microphage migration inhibitor factor. *Geometric mean values for measurement during and after surgery were adjusted for age, pathologic entity, preoperative values, and treatment.

TABLE E3. Biochemical marker release in patients with double ventricular anatomy

Variable	Measurement point	Geometric mean* Controlled reoxygenation	Geometric mean* Standard (hyperoxic)	Ratio	95% CI	*P* value
Troponin I						
	Preoperatively					
	10 min on CPB	0.43	0.40	1.10		
	30 min on CPB	1.88	1.25	1.48		
	10 min off CPB	19.17	20.66	0.91		
	4 h after CPB	18.75	19.86	0.93		
	24 h after CPB	10.98	10.10	1.10		
	Test for interaction between treatment and time					.7
	Treatment effect, pooled over all points			1.1	0.93-1.29	.31
8-Isoprostane						
	Preoperatively					
	10 min on CPB	5.05	9.67	0.51		
	30 min on CPB	5.23	9.77	0.54		
	10 min off CPB	5.29	10.46	0.50		
	4 h after CPB	2.58	3.98	0.65		
	24 h after CPB	1.24	2.80	0.44		
	Test for common ratio					.9
	Treatment effect, pooled over all points			0.52	0.47-0.6	<.01
IL-6						
	Preoperatively					
	10 min on CPB	0.35	0.35	0.98		
	30 min on CPB	0.22	0.25	0.87		
	10 min off CPB	6.15	2.25	2.75		
	4 h after CPB	40.14	41.32	0.95		
	24 h after CPB	65.16	61.27	1.05		
	Test for interaction between treatment and time					.46
	Treatment effect, pooled over all points			1.15	0.87-1.48	.3
IL-8						
	Preoperatively					
	10 min on CPB	7.37	7.43	1.00		
	30 min on CPB	8.17	9.76	0.83		
	10 min off CPB	22.33	26.00	0.87		
	4 h after CPB	39.11	34.76	1.12		
	24 h after CPB	37.06	34.35	1.07		
	Test for interaction between treatment and time					.29
	Treatment effect, pooled over all points			0.98	0.87-1.1	.61
IL-10						
	Preoperatively					
	10 min on CPB	8.65	16.15	0.54		
	30 min on CPB	15.59	16.16	0.95		
	10 min off CPB	122.35	170.96	0.72		
	4 h after CPB	87.07	113.23	0.78		
	24 h after CPB	32.99	33.20	1.00		
	Test for interaction between treatment and time					.6
	Treatment effect, pooled over all points			0.79	0.66-0.95	.01
C3α						
	Preoperatively					
	10 min on CPB	1046.64	1024.24	1.02		
	30 min on CPB	1187.34	1219.30	0.95		
	10 min off CPB	1391.82	1708.55	0.81		
	4 h after CPB	1277.29	1221.53	1.05		
	24 h after CPB	925.24	943.06	1.00		
	Test for interaction between treatment and time					.62
	Treatment effect, pooled over all points			0.85	0.78-1.05	.38

(Continued)

TABLE E3. Continued

Variable	Measurement point	Geometric mean*		Ratio	95% CI	*P* value
		Controlled reoxygenation	Standard (hyperoxic)			
Cortisol						
	Preoperatively					
	10 min on CPB	306.58	344.30	0.89		
	30 min on CPB	329.73	354.34	0.93		
	10 min off CPB	259.80	314.30	0.81		
	4 h after CPB	129.06	188.94	0.68		
	24 h after CPB	283.37	297.32	0.95		
	Test for interaction between treatment and time					.68
	Treatment effect, pooled over all points			0.85	0.78-0.93	<.01
S100						
	Preoperatively					
	10 min on CPB	382.20	490.88	0.78		
	30 min on CPB	721.49	807.65	0.89		
	10 min off CPB	1707.54	2293.36	0.74		
	4 h after CPB	370.17	346.07	1.07		
	24 h after CPB	174.64	242.67	0.71		
	Test for interaction between treatment and time					.4
	Treatment effect, pooled over all points			0.83	0.74-0.93	<.01
α-GT						
	Preoperatively					
	10 min on CPB	3240.35	4037.40	0.79		
	30 min on CPB	4066.82	4446.05	0.91		
	10 min off CPB	5815.27	8080.95	0.71		
	4 h after CPB	9091.89	8340.08	1.10		
	24 h after CPB	3627.80	3765.82	0.95		
	Test for interaction between treatment and time					.4
	Treatment effect, pooled over all time points			0.87	0.78-0.99	<.01
MIF						
	Preoperatively					
	10 min on CPB	12.97	14.63	0.87		
	30 min on CPB	19.77	19.86	1.00		
	10 min off CPB	48.69	49.26	1.00		
	4 h after CPB	15.01	18.51	0.81		
	24 h after CPB	11.00	10.98	1.00		
	Test for interaction between treatment and time					.8
	Treatment effect, pooled over all points			0.93	0.87-1.02	.11

CI, Confidence interval; *CPB*, cardiopulmonary bypass; *IL*, interleukin; *C3α*, complementary activation; *α-GT*, α-glutathione S-transferase; *MIF*, microphage migration inhibitor factor. *Geometric mean values for measurement during and after surgery were adjusted for age, pathologic entity, preoperative values, and treatment.